A CRYSTALLOGRAPHIC STUDY OF VERTEBRATE OTOLITHS

DIEGO CARLSTRÖM

Department of Medical Physics, Karolinska Institutet, Stockholm, Sweden

Although the physiological significance of the static organ is rather well established, little attention has been paid to the structure and composition of the dense bodies in the labyrinth of vertebrates. One exception is the work done on the well-known "ear-stones" of bony fishes. Ever since 1899, when Reibisch demonstrated that fish otoliths could be used for accurate age determinations, these bodies have been subjected to extensive studies. But on the whole, the information available regarding the nature of the calcareous deposits in the labyrinth of vertebrates other than teleosts is very meager.

It has long been known that the dense bodies in the inner ear occur either as solitary large "ear-stones" or as masses of minute particles, "ear-dust." In what follows, the former will be referred to as "statoliths," and the latter as "statoconia." As is customary, the term "otolith" is used in a broader sense to mean any type of a dense body in the labyrinth. It is true that the function of statoliths and statoconia is not exclusively a static one (Lowenstein, 1950), but we prefer the use of these well-known terms to the introduction of entirely new ones.

In the labyrinth of teleosts there are generally three large statoliths, each having an irregular unsymmetrical shape which is characteristic of the species. These bodies derive their names from their shape in carps: sagitta (arrow), lapillus (small stone), and asteriscus (star). They are located in sacculus, utriculus and lagena, respectively. Most other vertebrates, however, have otolith masses consisting of a very great number of small statoconia held more or less firmly together by an organic gel. The statoconia have microscopic dimensions, usually between 1 and 50 microns, are symmetrically shaped and are not characteristic for the species. Recently, otoliths having a size somewhere between statoliths and statoconia have been described by Frizzell and Exline (1958), who suggested the name "ossiculiths" (*ossiculum* bonelet + *lithos* stone) for such bodies. This term is rather unfortunate, not only because of the mixture of Latin and Greek, but especially because these bodies have nothing at all to do with bone. According to these authors, "ossiculiths" should be small (50–500 μ) plano-convex or irregularly shaped particles which are found occasionally with the ordinary statoliths of teleosts. However, in a high percentage of the individuals they occur in one of the labyrinths only, and are then often associated with malformed or abnormal statoliths (Weiler, 1959). "Ossiculiths" can thus hardly be regarded as normal formations in the teleost labyrinth. Irregularly shaped small bodies may, on the other hand, be found regularly together with statoconia, as in the labyrinth of the lamprey, *Lampetra* (Studnička, 1912). In the labyrinth of sturgeons, *Acipenser*, all stages in shape and structure between statoconia and statoliths are found. Some of these intermediate-sized bodies have evidently arisen by the coalescence of several smaller units. "Ossiculiths" are thus not characteristic of teleosts, and a better name and definition of these particles is needed. We suggest here the term "microstatolith" for any endogenous minute body of asymmetric shape found in the vertebrate labyrinth. In addition to the endogenous statoconia, some species of elasmobranchs also have exogenous minute bodies in their labyrinths. These grains, consisting of sea-sand, have entered the inner ear through the endolymphatic duct.

Regarding the composition of otoliths it is commonly stated (even in modern textbooks) that all kinds of vertebrates have otoliths consisting of calcium carbonate in its aragonite form. This is not true. By using x-ray diffraction, Brandenberger and Schintz (1945) found that statoconia of man and domestic fowl consisted of calcite, which is the other well-known polymorph of calcium carbonate. The occurrence of calcite in the labyrinth of a few other species of mammals and birds was later established by Carlström *et al.* (1953), Carlström and Engström (1955), and Sasaki and Miyata (1955). Calcite is also known to constitute the endogenous statoconia of the spined dogfish, *Squalus acanthias* (Vilstrup, 1951). In a crystallographic study of otoliths of 14 vertebrate species, Carlström and Engström (1955) detected still another mineral. The statoconia of the lamprey, *Lampetra fluviatilis,* were found to consist of calcium phosphate instead of calcium carbonate.

The aim of the present study was to make a microscopic and x-ray crystallographic analysis of otoliths from a representative collection of vertebrates. A survey of this kind would unveil the presence of any regularity in the distribution within different vertebrate groups of the different minerals occurring in the labyrinth. If such a regularity were found it would be of value in the classification of certain vertebrates. Moreover, a knowledge of the composition and the physical properties of otoliths should be of interest both from the crystallographic and the physiological point of view.

Material and Methods

The material consisted, if not otherwise stated, of otoliths from fresh adult specimens. After rapid washing in water to get rid of the organic jelly usually embedding the statoconia, the material was dried in air and analyzed as soon as possible. This procedure was necessary since some of the calcium carbonate polymorphs are known to convert quite rapidly to more stable forms, especially under humid conditions. The dried material could be stored for several years without noticeable alterations. The number of vertebrate species investigated was limited to 58. However, these were selected with an aim to cover as far as possible the whole subphylum of vertebrates.

The entire material was analyzed by ordinary powder x-ray diffraction. In cases where both statoliths and statoconia were present simultaneously, they were analyzed separately. The diffraction patterns were recorded with Ni-filtered Cu radiation in a Debye-Scherrer camera having a diameter of 114 mm. In a few instances, micro-diffraction techniques were also employed. Ordinary microscopy, as well as polarization microscopy, was used for the study of ground sections of statoliths and of the size, shape and texture of statoconia.

Here it should be mentioned that even if x-ray diffraction is a supreme method for the identification of crystalline materials, it does not yield any analytical information regarding the nature of non-crystalline materials. In the present study, only the crystalline part of otoliths was studied, and the amount and nature of such constituents as water and organic material were accordingly not determined. Only in one case a completely "amorphous" diffraction pattern was obtained. By adequate treatment of the actual otoliths, the inorganic component could be converted to a crystalline state suitable for x-ray diffraction.

Results

A summary of the results is presented in Figure 5, which also shows schematically the relation between the different vertebrate groups investigated. A detailed description is given below. The vertebrate species are arranged according to the classification of Young (1962).

Superclass Agnatha

Class Cyclostomata

The two still existing orders of this class are represented here by one species each: the lamprey, *Lampetra fluviatilis,* and the hag-fish, *Myxine glutinosa,* both of which have calcareous bodies in their labyrinths. Incidentally, the otoliths are the only calcified structures found in these animals.

In *Lampetra,* the otoliths consisted mainly of statoconia in the form of transparent spheres showing a concentric layering. Their sizes usually ranged from 2 to 25 μ in diameter but were occasionally larger. Quite frequently two or three statoconia were fused together, as illustrated in Figure 1a. In addition a few larger bodies (100–250 μ) were found in each labyrinth. The largest of these had a characteristic, somewhat plano-convex shape and a regular layered structure, as seen in Figure 1b. These microstatoliths were apparently not formed by fusion of a large number of statoconia as suggested by Studnička (1912). X-ray diffraction patterns of statoconia, as well as of the microstatoliths, gave nothing but a broad halo, showing that the mineral salt composing these bodies was in a non-crystalline state. After heating to 700° C., a treatment which causes recrystallization, perfectly sharp patterns of apatite, a basic calcium phosphate, were obtained. Upon solution of fresh otoliths of *Lampetra* in acid, a slight effervescence was observed, indicating that these bodies also contained some carbonate.

In the labyrinth of *Myxine* only statoconia were found. They had the same shape and about the same size as in *Lampetra;* an apatite diffraction pattern was obtained in this case without previous heating. The diffraction lines were, however, very broadened, showing that the degree of crystallinity was fairly low. Since the presence of carbonate hinders the crystallization of apatite, it is inferred that the carbonate content of statoconia of *Myxine* probably is much less than in otoliths of *Lampetra*. This explains why Retzius (1881) did not observe any gas-bubbles upon dissolving in acids the statoconia from *Myxine*.

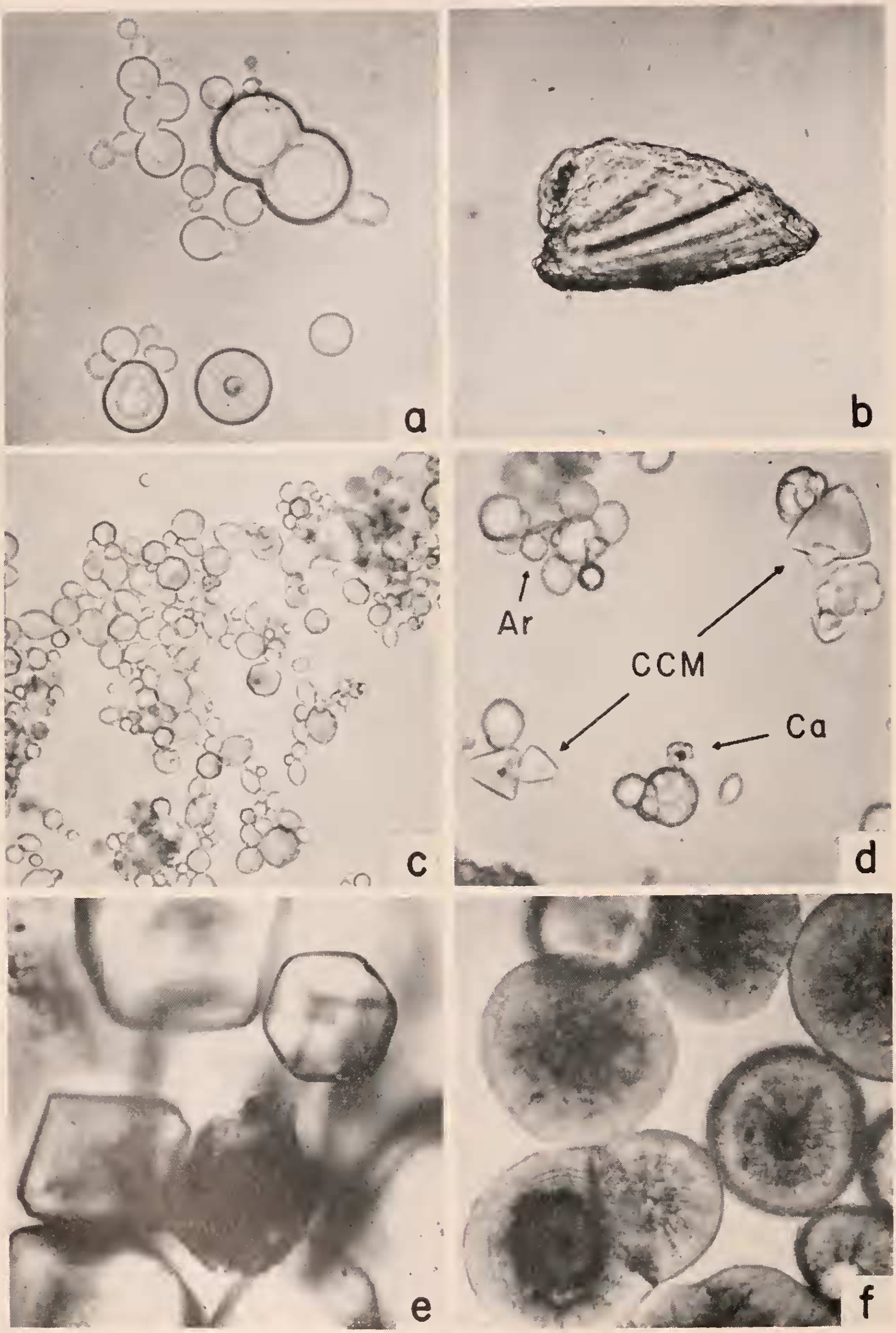

FIGURE 1.

Superclass Gnathostomata

Class Elasmobranchii

In the subclass Selachii, the otoliths of the following 9 species, including 6 sharks and three rays, were investigated: the porbeagle, *Lamna cornubica;* the bull-shark, *Carcharhinus leucas;* the lemon-shark, *Negaprion brevirostris;* the nurse-shark, *Ginglymostoma cirrhatum;* the tiger-shark, *Galeocerdo cuvier;* the spined dog-fish, *Squalus acanthias;* the starry ray, *Raja radiata;* the thornback ray, *Raja clavata;* and the common skate, *Raja batis.* The four first-mentioned sharks and the three rays had statoconia of a rather similar appearance. Usually, they were lemon-shaped, having a rounded or slightly hexagonal cross-section; almost spherical or quite elongated statoconia were also frequently observed. A typical example is shown in Figure 1c. The size varied from a few up to about 40 microns, with a mean size of roughly 10 μ. In polarized light, the statoconia were highly birefringent but each statoconium did not show perfect extinction, indicating that they did not consist of single crystals. This was also evident from the x-ray diffraction analyses. Diffraction patterns of stationary specimens gave no "spottiness" of the diffraction lines, in spite of the relatively large particle size, which means that each statoconium was a heavily distorted single crystal or rather a polycrystalline body. In all these cases, the diffraction patterns showed the statoconia to consist of pure aragonite. In the case of the tiger-shark, on the other hand, the microscopic investigation showed that besides statoconia of the type described above, two additional crystalline materials were present. One type, which was less frequent, consisted of small irregularly shaped particles apparently made up by clusters of spherites, while the other type, which constituted about one third of the total number of statoconia, formed perfect single crystals having the shape of trigonal bipyramids. The latter were also highly birefringent and ranged in size from a few up to 20 microns. In addition to aragonite lines, the powder diffraction pattern of statoconia from the tiger-shark showed faint calcite lines and fairly strong lines of a substance which could not be recognized as any known mineral deposit of biological origin. In diffraction patterns of stationary specimens, the lines from this unknown substance were easily distinguished from the aragonite and calcite lines because of their "spottiness." Hence, they were caused by the trigonal single crystals observed microscopically.

From the x-ray diffraction pattern, the trigonal crystals could be identified as calcium carbonate monohydrate ($CaCO_3 \cdot H_2O$), a substance which to our knowledge never has been encountered in nature before. A photomicrograph of statoconia from a tiger-shark, showing the three different calcium carbonates present, is shown in Figure 1d.

The statoconia of the spiny dog-fish, on the other hand, showed no similarities

FIGURE 1. Otoliths of cyclostomes and elasmobranchs: Statoconia (a) and a microstatolith (b) of the lamprey, *Lampetra*. Figures (c)–(f) show statoconia of the thornback ray, *Raja;* the tiger-shark, *Galeocerdo;* the spined dog-fish, *Squalus;* and the rat-fish, *Chimaera*, respectively. In (d) three different minerals can be distinguished: Ar = aragonite, Ca = calcite, CCM = calcium carbonate monohydrate. Note the dark quartz grain in (e) among rhombic calcite crystals. Magnification: (a) and (c)–(f) 750 ×, (b) 200 ×.

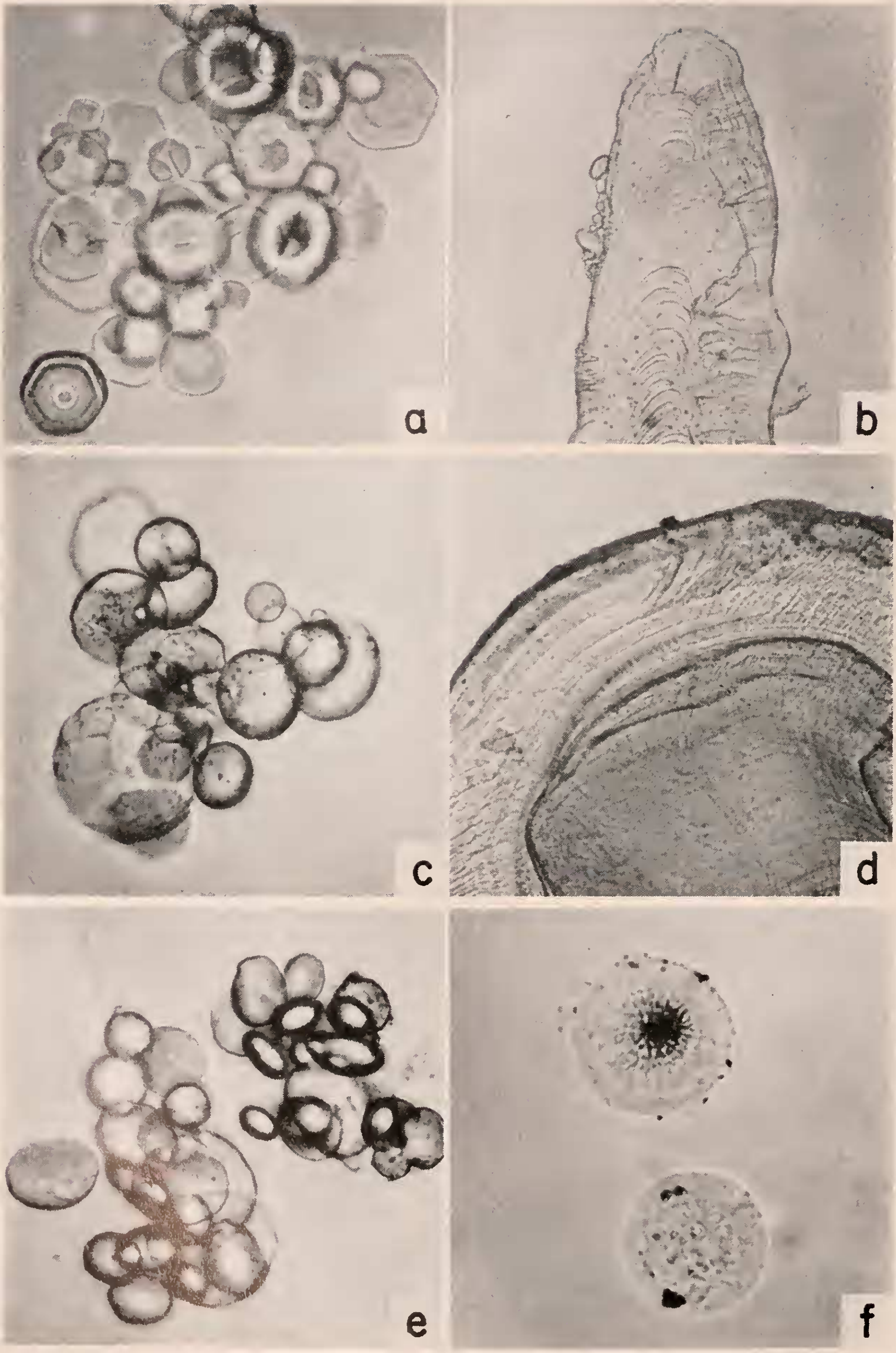

Figure 2.

to those already described. Three specimens investigated all had statoconia of both endogenous and exogenous origin, but the relative amounts of the two types varied in different specimens. The endogenous statoconia were quite large (some 30–150 μ), forming water-clear rhombohedral single crystals which, from the x-ray diffraction analysis, were found to consist of pure calcite (Fig. 1e). The exogenous grains, on the other hand, had very irregular shapes and varied from colorless and transparent to black and opaque. Since they were insoluble in weak acids, they could easily be separated from the endogenous calcite statoconia. X-ray diffraction patterns of samples treated in this manner showed the exogenous bodies to consist mainly of α-quartz (SiO_2) plus minor amounts of ilmenite ($FeTiO_3$), *i.e.*, sea-sand. It is well-known that some elasmobranchs, presumably those having a wide ductus endolymphaticus, may use sea-sand as statoconia. Thus, Retzius (1881) observed irregular grains in the labyrinth of *Acanthias;* and Stewart (1903–1906) seems to have been the first to identify these particles as sand. Exogenous statoconia are also known to occur in *Pristiophorus japonicus* (Iseltöger, 1941), *Rhina squatina* (Stewart, 1903–1906; Nishio, 1926), *Torpedo ocellata* (Nishio, 1926; Werner, 1930), and *Torpedo marmorata* (Werner, 1930). While some authors state that the statoconia of these species consist of nothing but sand, others have found both sand and endogenous statoconia or only endogenous statoconia in the same species. This discrepancy may be due partly to varying proportions of the two constituents in different specimens, but it may also depend on the use of preserved material in which the calcite particles may have disappeared. In the case of *Rhina squatina*, however, it is possible that this shark depends entirely on statoconia made up by foreign particles. When investigating a mature embryo of this species, Stewart found no statoconia at all, while in an unborn *Squalus acanthias* used for comparison there was an abundance of endogenous statoconia. Whether preserved or fresh material was used by Stewart is not known, and his observation certainly needs confirmation.

In the subclass of Bradyodonti, the rat-fish, *Chimaera monstrosa*, was investigated. Its statoconia consisted of almost perfect spherites of a brownish tint showing a concentric lamellar structure as well as a radial striation. Their diameter was quite large (20–100 μ), as seen in Figure 1f. The x-ray diffraction patterns showed them to consist of pure aragonite. Such spherites of aragonite were not found in any other vertebrate species during this investigation, but they are probably not unique for *Chimaera*. According to Nishio (1926), the statoconia of the shark, *Heptanchus cinereus*, are concentrically layered spheric bodies of about the same size as were here found in *Chimaera*. In *Heptanchus*, these statoconia are reported to be cemented together by an amorphous calcareous material, thereby virtually forming a kind of statolith.

Class Actinopterygii

In the superorder Chondrostei, the otoliths of two sturgeons, *Acipenser sturio* and *Acipenser güldenstädti*, and of two species of bichir, *Polypterus ornatipinnis*

FIGURE 2. Otoliths of Chondrostei and Holostei: Statoconia (a) and a ground section of a statolith (b) of a sturgeon, *Acipenser*. Figures (c) and (e) show statoconia from two species of bichir, *Polypterus delhezei* and *P. ornatipinnis*. A ground section of a statolith from the former in (d). Statoconia of the bow-fin, *Amia*, in (f). Magnification: Statoliths 200 ×, statoconia 750 ×.

and *Polypterus delhezei,* were investigated. In accordance with the findings of Retzius (1881), Shepherd (1914), and Weiler (1959), both statoconia and statoliths were found in the labyrinth of *Acipenser sturio.* This feature seems to be general in sturgeons, since it here was found also in *Acipenser güldenstädti* and is reported in *Acipenser ruthenus* (Weiler, 1959). In addition, microstatoliths—*i.e.*, small irregularly shaped bodies—were found, in accordance with the observation by Weiler (1959). The statoconia showed quite a variation in size, from a few up to 100 microns. The smaller statoconia generally were of a rounded shape with an indication of a hexagonal outline, whereas the larger ones were more disk-shaped. In these latter, concentric hexagonal lamellae were observed (Fig. 2a). The optical examination, as well as the x-ray diffraction, showed that the statoconia in spite of their regular polyhedral shape were not perfect single crystals. The extinction position was, however, fairly sharp, and good conoscopic interference figures could be obtained, indicating that the sub-units building up the statoconia must be well aligned. The statoliths, on the other hand, were definitely polycrystalline and had the usual lamellar structure found in statoliths of teleosts (Fig. 2b). The x-ray diffraction patterns showed that all types of bodies found in the labyrinth of *Acipenser sturio* consisted of pure vaterite, the third and most unstable modification of anhydrous calcium carbonate. In the case of *Acipenser güldenstädti* the diffraction patterns, besides the dominating vaterite patterns, showed faint lines of aragonite and calcite. However, this specimen had been preserved in formalin for several months and the presence of these two minerals was probably an artifact. Both the aragonite and the calcite might have been produced by transformation of vaterite.

The two *Polypterus* species here investigated both had well-developed statoliths. Regarding the fine structure, the statoliths resembled completely those of teleosts. However, in both species small amounts of statoconia were also found in the labyrinth, side by side with the statoliths, a feature which seems to have been overlooked by earlier investigators. The statoconia were lens-shaped with a diameter of about 5–40 microns and showed no evident internal structure. Photomicrographs of a thin section of a statolith, as well as of statoconia, are shown in Figures 2c, d, and e. The x-ray crystallographic examination showed that while the statoliths were composed of pure aragonite, the statoconia consisted of pure vaterite.

In the superorder Holostei two species, the bow-fin, *Amia calva*, and the gar-pike, *Lepisosteus osseus*, were investigated. The otoliths of the latter came from a whole dried head. The static bodies of both species very much resembled those of *Polypterus*, *i.e.*, they consisted of statoliths of the ordinary teleost type as well as of thin, lens-shaped statoconia often showing a faint radiating structure. The composition of these otoliths was the same as in the case of *Polypterus*, with statoliths consisting of pure aragonite and statoconia consisting of pure vaterite.

In the superorder Teleostei the otoliths of 13 species were investigated. The material consisted of herring, *Clupea harengus;* salmon, *Salmo salar;* pike, *Esox lucius;* catfish, *Corydoras aeneus;* common eel, *Anguilla anguilla;* needlefish, *Belone belone;* platy, *Xiphophorus maculatus;* cod, *Gadus callarias;* haddock, *Gadus aeglefinus;* bearded cottus, *Agonus cataphractus;* perch, *Perca fluviatilis;* sole, *Solea solea*, and angler, *Lophius piscatorius*. As could be expected, only statoliths were found in all species. In the vast literature on the static bodies of teleosts, there seems to be only one exception to the common rule that teleosts possess three well-defined and characteristic bodies in each labyrinth. According to Thompson (1888) the sun-fish, *Mola mola*, should possess statoconia instead of statoliths.

The microscopic structure of the teleost statoliths is well-known and needs no elaboration here. After the careful investigation by Irie (1960), using microradiography, electron microscopy, and x-ray diffraction, there is not much to add regarding the structure of these polycrystalline bodies.

One of the first chemical analyses of teleost statoliths was carried out by Wicke (1863) who found that the statoliths of cod consisted of 91.1% inorganic and 8.9% organic material. The inorganic salt was found to be calcium carbonate admixed with small amounts of magnesium carbonate, calcium phosphate and calcium sulphate. On the basis of optical data, Immermann (1908) suggested that the calcium carbonate should be in its aragonite modification, a proposal which has been fully confirmed by later investigations. In the present material no other crystalline component than aragonite was found. This is in perfect accordance with the few earlier x-ray crystallographic investigations made on teleost statoliths. Brandenberger and Schintz (1945), Sasaki and Miyata (1955), and Irie (1955) investigated the statoliths of *Esox lucius*, *Scomber scombrus* and *Pseudosciaena manchurica*, respectively. Aragonite was found to be the only crystalline material in these cases. The statement by Lunde (1929) that statoliths of cod, in addition to $CaCO_3$, water and organic material, should also contain 11.44% CaO therefore seems quite surprising. It is not only improbable that calcium oxide should occur in a non-crystalline state, thereby escaping detection with x-ray diffraction, but it is quite impossible for this compound to form and to remain stable in the presence of water. Not only are the statoliths surrounded by an aqueous medium *in vivo* but they also contain some water at crystal interfaces and in the organic phase.

Occasionally, abnormal statoliths are found in teleost labyrinths. The structure and composition of these bodies was not investigated but it seems probable that the calcium carbonate in abnormal statoliths may occur in another modification than the ordinary aragonite. This is not yet proven, but at least in one case reported by Immermann (1908) an abnormal statolith of plaice, *Pleuronectes platessa*, seemed to be composed of calcite.

Class Crossopterygii

This class is represented here by the only living coelacanth, *Latimeria chalumnae*, and by one dipnoid, the Congo lung-fish, *Protopterus dolloi*. The *Latimeria* material consisted of the right sagitta from two individuals, the second and the seventeenth specimens caught. Because of the rarity of this material a more detailed description will be given here. The statolith of the second *Latimeria* was quite large, forming an almost circular, concavo-convex plate with a rounded edge. The average diameter was about 21 mm. and the average thickness about 4 mm. The convex surface was rather smooth while the slightly concave surface showed concentric lamellae, as well as a rough tubercular central part (Figure 3a and b). The weight was 2683 mg. and the density 2.775. A microradiogram showed internal cracks radiating from the center. Since these cracks were not

FIGURE 3.

filled by the immersion liquid used for the density determination, the figure obtained was certainly a trifle too low. In the uneven central area a few very small and water-clear crystals having well-developed faces were observed. Since the statolith had been lent on the condition that it should not be destroyed, material for the x-ray diffraction analysis was taken from the surface and from a depth of 1 mm. through a 0.5-mm.-wide drill hole. The small transparent single crystals were found to consist of pure calcite, whereas the sub-surface sample, as well as material taken from different sites on the surface, consisted of pure aragonite. The statolith of the seventeenth *Latimeria* was very similar to that already described. Its average diameter was slightly less, around 19 mm., but it was strikingly thinner, resulting in a weight of only 1428 mg. The central tubercular area of this statolith was quite pronounced and consisted to a large extent of calcite. The rest of the statolith was apparently made up of aragonite with the exception of a thin yellowish crust covering large parts of the surface. This crust proved to consist of apatite which most probably had been deposited during the preservation. The presence of calcite in both specimens might or might not have been produced *in vitro* but the finding of small amounts of mineral other than aragonite does not interfere with the over-all picture of the *Latimeria* statolith as being of the typical teleost type. According to Anthony (personal communication), the labyrinth of *Latimeria* seems not to contain any other dense bodies than the single, large sagitta. However, detailed information on the anatomy of the inner ear of *Latimeria* is still missing and we do not therefore know if, besides the statolith, there are also statoconia present, as is the case in Chondrostei and Holostei.

In the labyrinth of the lung-fish, *Protopterus dolloi*, only statoconia were found. They were spindle-shaped with pointed ends (Fig. 3c), having lengths varying from about 1 micron or less up to 100 microns. It is well established that all Dipnoi have statoconia in their labyrinths (Retzius, 1881). According to Weiler (1959), *Neoceratodus* and *Protopterus* also possess microstatoliths. While Shepherd (1914) found statoconia in *Lepidosiren paradoxa*, he claims that the static bodies of *Neoceratodus forsteri* consist of two statoliths, namely, lapillus and sagitta, but he remarks (p. 106) that they "are very chalky-looking." This finding is certainly a misinterpretation, possibly due to the use of preserved instead of fresh material.

Optical as well as x-ray diffraction examination showed that, in contrast to the finding in all other fishes, the statoconia of *Protopterus* consisted of perfect single crystals of aragonite. Such statoconia are otherwise only encountered in the classes of Amphibia and Reptilia.

Class Amphibia

No member of the subclass Apoda was examined.

In the subclass Urodela, three species were investigated, namely the European salamander, *Salamandra maculosa*; the smooth newt, *Triturus vulgaris*, and the

FIGURE 3. Otoliths of Crossopterygii and amphibians: Statolith of *Latimeria* seen from its convex (a) and concave (b) side. Statoconia of the Congo lung-fish, *Protopterus* (c): the European salamander, *Salamandra* (d); the clawed toad, *Xenopus* (e); and the common frog, *Rana* (f). Magnification: (a) and (b) 3.6 ×, (c)–(f) 750 ×.

FIGURE 4.

axolotl, *Amblystoma mexicanum.* Of the last mentioned species, only formalin-fixed material was available. In all cases the statoconia were elongated particles with pointed ends having lengths of 3–40 μ (Fig. 3d). They consisted of single crystals of pure aragonite. This seems to be in conflict with the finding of Hastings (1935), who from x-ray diffraction as well as chemical analyses reported the occurrence of some 20% of calcium phosphate (apatite), together with aragonite, in otoliths of *Amblystoma tigrinum.* The explanation of this observation is that the axolotl has a most unusual kind of otholith, consisting of densely packed statoconia surrounded by a thin, almost transparent shell. Diffraction patterns of this shell during the present investigation showed it to consist of poorly crystalline apatite. It is not known whether such composite "statoliths" occur in other larval urodeles, but Sarasin and Sarasin (1890), examining the species *Ichthyophis glutinosus* belonging to the subclass Apoda, found that the embryos possessed a single rounded statolith in each labyrinth whereas adult specimens had the usual arrangement found in Amphibia, *i.e.*, a great number of statoconia. The statoliths of the embryos may possibly have had the same structure as larval *Amblystoma.* Further investigations on urodeles are needed to clarify the function of the composed "statoliths" of the larval stage and their relation to the statoconia of adult animals.

In the subclass Anura, the material came from the following species: the common toad, *Bufo bufo;* the common frog, *Rana temporaria,* and the clawed toad, *Xenopus laevis.* In all these cases, the labyrinth bodies, as well as the content in the endolymphatic sacs, consisted of elongated single aragonite crystals having tapered ends. The statoconia of *Rana* had well-developed crystal faces while the statoconia of *Bufo* and *Xenopus* were more spindle-shaped (Figs. 3e and f) and resembled the statoconia of the Congo lung-fish (Fig. 3c).

The otoliths of frogs have been analyzed earlier by means of x-ray diffraction. In accordance with the above findings, pure aragonite patterns have always been obtained (Funaoka and Toyota, 1928; Brandenberger and Schintz, 1945; Carlström and Engström, 1955; Sasaki and Miyata, 1955).

Class Reptilia

The subclass Anapsida was represented by two species: a European tortoise, *Testudo* sp., and the European water-tortoise, *Emys orbicularis.* In both, the labyrinth contained statoconia and these were rounded or elongated. While the statoconia of *Testudo* had an average size of about 10 microns, those of *Emys* were smaller, the average being only about 5 microns. The x-ray diffraction patterns showed typical aragonite diagrams but, in addition, rather strong calcite lines were present. The aragonite-to-calcite ratio was estimated to 3:1.

In the subclass Lepidosauria, the static bodies of four species were analyzed: the slow-worm, *Anguis fragilis,* a monitor lizard, *Varanus flavus,* the grass-snake, *Natrix natrix,* and the adder, *Vipera berus.* All species possessed statoconia of about the same size and shape: elongated single crystals with pointed ends having

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FIGURE 4. Otoliths of reptiles, birds and mammals: Statoconia of the common grass snake, *Natrix* (a); pigeon, *Columba* (b); the carpercaillie, *Tetrao* (c); the opossum, *Didelphis* (d); the porpoise, *Phocaena* (e); and sheep, *Ovis* (f). Magnification 750 ×.

lengths of some 1–50 microns (Fig. 4a). The diffraction patterns showed aragonite diagrams on which faint calcite lines were superimposed, except in *Varanus* in which the statoconia gave a perfectly pure aragonite pattern.

No fresh material was available from the subclass Archosauria.

Class Aves

In the subclass Neornithes, containing the three recent superorders Paleognathae, Impennae, and Neognathae, only the last mentioned was represented in this investigation. The static bodies of the following species were analyzed: pigeon, *Columba domestica;* domestic fowl, *Gallus domesticus;* the capercaillie, *Tetrao urogallus;* and the magpie, *Pica pica.* These species had statoconia of the usual form, *viz.,* elongated particles with pointed ends having an average length of about 10–20 microns. The x-ray crystallographic analysis yielded diagrams of pure calcite and the microscopic examination showed the statoconia to consist of perfect single crystals.

From an optical examination, Herzog (1925) claimed that the statoconia of domestic fowl consisted of aragonite. However, by means of x-ray diffraction, Brandenberger and Schintz (1945) could later show that they consisted of calcite.

Class Mammalia

No member of the subclass Prototheria was available. In the subclass Theria, on the other hand, both the infraclasses Metatheria and Eutheria were repre-

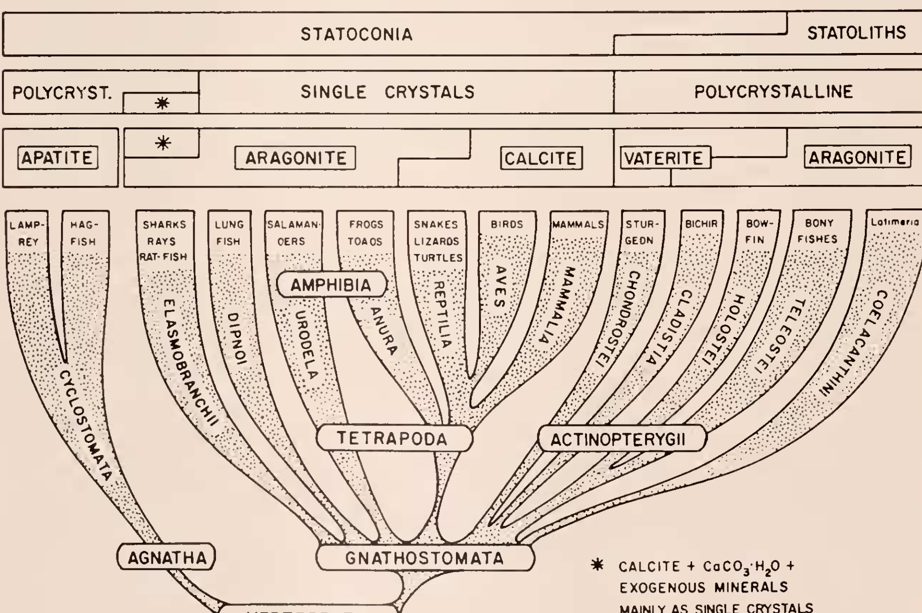

FIGURE 5. A schematic drawing showing the present knowledge regarding the distribution of statoliths and/or statoconia, their texture and mineral composition throughout the vertebrate series.

sented. The material from the former consisted of otoliths from the opossum, *Didelphis marsupialis*, while the material from the latter came from: the common shrew, *Sorex araneus;* man, *Homo sapiens;* guinea-pig, *Cavia porcellus;* rabbit, *Oryctolagus cuniculus;* cat, *Felis domesticus;* the gray seal, *Halichaerus grypus;* sheep, *Ovis aries;* and the porpoise, *Phocaena phocaena*.

All mammals investigated possessed elongated statoconia with pointed ends (Fig. 4d–f). They usually were quite small, rarely exceeding 10 microns in length; but in the labyrinth of the seal and the opossum, particles up to 20 μ in length, were occasionally observed. The latter species also had much larger particles consisting of an intergrowth of several statoconia. The statoconia were found to be single crystals. In all cases, they provided x-ray diffraction patterns of pure calcite. This finding is in agreement with earlier x-ray crystallographic analyses on statoconia of *Homo* carried out by Brandenberger and Schintz (1945), Carlström *et al.* (1953), and Sasaki and Miyata (1955). Besides human statoconia, the latter authors also investigated statoconia from guinea-pig, rabbit and rat. In all cases calcite patterns were obtained.

Discussion

A. *Crystallographic aspects*

In the present study, two essentially different calcium salts, calcium phosphate and calcium carbonate, were found to compose the otoliths of vertebrates.

Calcium phosphate was found only in cyclostomes, where it existed in a non-crystalline or poorly crystalline state. In the latter case it had an apatite structure. Apatite is a mineral which is rarely encountered in calcareous deposits of invertebrates, but it is the most common mineral of vertebrates, since it is the main constituent of their teeth and bones. Biogenic apatite is usually a slightly impure hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. In vertebrate skeletons it is always cryptocrystalline, *i.e.*, the apatite crystals are of colloidal size. The main "impurity" is carbonate, usually 1–5% CO_2. However, the carbonate does not form a separate calcium carbonate phase but is somehow associated with the apatite. There seems to be a proportionality between the carbonate content and the degree of crystallinity. From *in vitro* experiments it has been shown that the presence of carbonate disturbs the crystallization of apatite (Trautz, 1960). In the present material it was not possible to determine the CO_2 content of the cyclostome otoliths because of the small amount of material available. However, from a comparison with the degree of crystallinity of biogenic apatites with known carbonate content, it is inferred that the statoconia of *Myxine* should contain less than 5% CO_2, whereas the carbonate content of the otoliths of *Lampetra* probably is in the order of 10%.

Calcium carbonate was found to constitute the statoliths and statoconia of all other vertebrates investigated, that is, all gnathostomes. Calcium carbonate is extremely common as skeletal material in invertebrates but is not reported to occur in vertebrates except in otoliths, in egg-shells of birds, and in some pathological deposits. Anhydrous calcium carbonate ($CaCO_3$) is known to crystallize in three different polymorphs, namely, as calcite, aragonite, and vaterite. While calcite belongs to the rhombohedral system, both aragonite and vaterite crystallize in the orthorhombic system. Vaterite, however, is pseudohexagonal. In all three polymorphs the planar CO_3-groups have their planes mutually parallel, an arrangement which explains the very high birefringence of these carbonates.

Under ordinary conditions calcite is the stable form. Although aragonite is metastable, its transformation to calcite is usually very slow. Vaterite, on the other hand, is quite unstable, especially when in contact with water; when dry it may stay unaltered for several years. All the calcium carbonate polymorphs can be artificially produced and a mixture of the three is usually obtained *in vitro* when precipitated from concentrated solutions. Calcite is often the predominating one and is also most easily obtained in pure form. It is well-known that elevated temperature (30–70° C.), as well as the presence of small amounts of Mg^{+2}, Sr^{+2}, Ba^{+2}, Pb^{+2} or SO_4^{-2}, favor the formation of aragonite. Low temperatures, on the other hand, seem to favor the precipitation of vaterite, but this polymorph is rather difficult to get totally free from contamination by the other two. The chemical and physical conditions which regulate the formation of the different $CaCO_3$ polymorphs *in vitro* seem not to be known in detail. Since the physico-chemical conditions of the endolymph are even less known, it is not possible to say why one or the other calcium carbonate polymorph is produced *in vivo*. It is, however, interesting to note that the calcium carbonate modifications of otoliths usually are in an extremely pure state. Only in a few instances was a mixture of two polymorphs encountered in the same labyrinth. It is of special interest that two different crystal forms of $CaCO_3$ can exist in a perfectly pure state side by side, one forming the statoliths and the other forming the statoconia. This was the situation in the labyrinths of *Polypterus, Lepisosteus,* and *Amia*.

Regarding the occurrence of the three calcium carbonate polymorphs in nature, calcite is by far the most common. Not only is it a rock-forming mineral, but it also constitutes the inorganic salt of the skeletons of many invertebrates, such as Foraminifera, echinoderms and crustaceans (Chave, 1945). Aragonite, although quite rare as a mineral, is also common in biogenic deposits; scleractinian and massive octocorals and hydrocorals, for example, have skeletons consisting of extremely pure aragonite (Chave, 1954; Wainwright, personal communication). A mixture of the two is also common: mollusc shells and pearls are built up in layers of calcite and aragonite crystals (Wilbur, 1960). The third $CaCO_3$ polymorph, vaterite, seems, on the other hand, to be exceedingly rare in nature. It is reported to occur, together with calcite and aragonite, in the repair tissue of gastropod shells (Mayer, 1931) as well as in pathological calcifications such as urinary calculi and bile stones (Lagergren, 1962). To our knowledge, however, it has never before been found as the only crystalline component in a calcareous deposit. The occurrence of pure vaterite in the labyrinth of some fishes indicates the presence of a component stabilizing vaterite *in vivo*.

Besides the polymorphs already mentioned, which are usually well-crystallized in biogenic products, calcium carbonate may also occur in a non-crystalline state. In analogy with the situation for calcium phosphate, the crystallization of calcium carbonate is disturbed by presence of phosphate, resulting in amorphous or poorly crystallized material. Such non-crystalline deposits, mainly consisting of calcium carbonate, are known to occur in biological calcifications. Thus gastroliths, as well as large parts of the carapaces of many crustaceans, consist of amorphous calcium carbonate admixed with small amounts of phosphate. Whether this type of calcium carbonate is hydrated seems not to be known. From the present investigation there is no indication that non-crystalline $CaCO_3$ occurs in otoliths. It may, however, be a transitory step during their formation.

The finding in the present study of a well-crystallized hydrated calcium carbonate, $CaCO_3 \cdot H_2O$, as a biogenic product is certainly unique. This calcium carbonate was until recently unknown. In 1950, Brooks *et al.* succeeded for the first time in identifying the monohydrate which they prepared synthetically. A detailed description of its crystallographic properties is given by Lippmann (1959), who synthesized single crystals up to 0.1 mm. in size. Since this compound may be encountered in other biogenic deposits consisting of calcium carbonate, its x-ray diffraction characteristics are given in Table I. The unit cell dimensions found (a = 6.100 Å, c = 7.553 Å) are in good agreement with those given by Brooks *et al.* (1950) : a = 6.15 Å, c = 7.61 Å, and by Lippmann (1959) :

Table I

Powder diffraction data of biogenic $CaCO_3 \cdot H_2O$

Indexing on a hexagonal pseudocell (a = 6.100 Å, c = 7.553 Å) to d-values not smaller than 1.75 Å. Relative intensities: vs = very strong, s = strong, ms = medium strong, m = medium, w = weak, vw = very weak. Reflections with smaller d-values than those listed are all w or vw.

Index (hkl)	Spacing in Å		Intensity	Index (hkl)	Spacing in Å		Intensity
	$d_{calc.}$	$d_{obs.}$			$d_{calc.}$	$d_{obs.}$	
001	7.55	—		112	2.373	2.372	w
100	5.29	5.30	w	103	2.273	2.271	vw
101	4.33	4.34	vs	202	2.164	2.165	ms
002	3.78	—		210	1.997	1.997	w
102	3.07			113	1.942	1.942	w
	}	3.07	s				
110	3.05			211	1.930	1.929	s
111	2.827	2.829	m	004	1.889	—	
200	2.641	—		203	1.823	1.823	w
003	2.517			104	1.778	1.778	w
	}	2.510	vw				
210	2.493			212	1.765	1.765	w

a = 6.13 Å, c = 7.54 Å. The small differences observed are probably due to slightly different composition of the $CaCO_3 \cdot H_2O$ samples investigated. The above dimensions, according to Lippmann, relate to a hexagonal pseudocell. The true unit cell has an a-axis = $a\sqrt{3}$.

It should also be pointed out that the external shape of the single crystals of aragonite and calcite occurring as statoconia in land-vertebrates cannot serve for the identification of these minerals. Irrespective of the nature of the mineral, the single-crystal statoconia are almost invariably spindle-shaped. If crystal faces are developed they are usually too poor for a precise identification. In fact, the erroneous statement by Herzog (1925) that the statoconia of domestic fowl consist of aragonite was based on measurements of the angles between crystal faces.

B. *Biological aspects*

Since some striking differences in the occurrence and crystallographic properties of statoconia and/or statoliths were found between different groups of vertebrates (see Fig. 5), the possible biological significance of these differences needs a closer consideration. Our suggestions cannot, however, be regarded as having a general validity until more extensive material has been examined. It is fully realized that chemical relationships *per se* have a rather restricted value. The very same and highly specialized compound may be found in biological material of vastly different origin. To take an example, the respiratory blood pigment, haemoglobin, consisting of haeme-groups linked to polypeptide chains (which may vary in composition), is common to all vertebrates. However, it is also found in some annelids and crustaceans and in one or two species of insects and molluscs. In addition, it has been detected in a few plants (Fox, 1949). In the case of the vertebrate labyrinth, however, the situation is somewhat different. Not only are the otoliths homologous formations but their physiological role is almost identical throughout the vertebrate series. The sensitivity to gravitational force and linear acceleration is indeed a very primitive property in the animal kingdom. Moreover, these forces remain the same irrespective of the medium surrounding the animal. It can be inferred, therefore, that the otolith organ, when once developed, should be unaffected by such alterations in the outer environment as the step from an aquatic to a terrestrial life.

The anatomical division of vertebrates into two main groups, the Agnatha and the Gnathostomata, is reflected in the composition of their otoliths. Two chemically different minerals were found in the two groups, suggesting entirely different mechanisms during the formation of their static bodies.

When looking at the systematic distribution of the various calcium carbonates deposited in the labyrinth of gnathostomes, a general trend is discernible. Thus, aragonite and vaterite are found exclusively in cold-blooded vertebrates, whereas all warm-blooded vertebrates have otoliths consisting of calcite. Calcite was also found in the labyrinth of two sharks; the possible reason for this will be considered below. In reptiles, which form the bridge between warm- and cold-blooded vertebrates, the otoliths consisted generally of a mixture of aragonite and calcite. There is no explanation at hand for this general distribution of the calcium carbonate polymorphs. The body temperature alone cannot possibly be the main reason for the precipitation of one or the other form of carbonate.

A more detailed inspection of the results obtained from the different classes of gnathostomes reveals some interesting features. In elasmobranchs a unique situation is encountered. This is the only class in which the labyrinth is in contact with the outer environment through the endolymphatic duct. It is obvious that such a connection may influence the composition of the endolymph. However, the function of this passage *in vivo* is not established. We know that foreign particles, and thus also sea water, do enter the labyrinth of some species; but it is not known whether the endolymphatic duct of those species which possess only endogenous statoconia will also allow sea water to pass into the labyrinth. Clearly, differences in the local ionic environment in which the otoliths are formed and suspended may result in differences in their composition. It is worth mentioning that in those species of sharks and rays in which only endogenous statoconia were found, these were all very similar, consisting of minute aragonite bodies of a polycrystalline nature. In one species, additional statoconia, consisting of calcium carbonate monohydrate and calcite, were found. In the only elasmobranch here investigated, which certainly possessed an open ductus endolymphaticus, allowing sand to enter the labyrinth, the endogenous statoconia were completely different from those of the other elasmobranchs. On the whole, the elasmobranchs thus show a considerable heterogeneity in the crystallographic properties of their endogenous statoliths. It is anticipated that when more elasmobranch species are investigated, still other minerals may be encountered, owing to the role of the endolymphatic duct in ionic transport.

In the class of Actinopterygii, all species investigated belonging to the superorders Chondrostei and Holostei had statoconia composed of vaterite, a mineral which was not found in the labyrinth of any other group of vertebrates. Moreover, the statoconia always occurred together with large statoliths, another unique feature among gnathosotomes. In these respects, the Chondrostei and Holostei seem to form a perfectly homogeneous group. However, in this group, the sturgeons deviate from the other species in that their statoliths and microstatoliths consist of vaterite, while the statoliths of *Polypterus*, *Amia* and *Lepisosteus* are of the common teleost type, that is, of pure aragonite. It appears, therefore, that *Polypterus* is chemically closer related to *Amia* and *Lepisosteus* than to *Acipenser*. The rest of the actinopterygian fishes investigated, *i.e.*, all the teleosts, had statoliths of the same structure and composition, thereby forming a completely homogeneous group. It is commonly realized that the classification of actinopterygians is far from satisfactory. To take an example, the correct place of *Polypterus* in the system of fishes is much debated. In Figure 5, it has been placed all by itself in the group Cladistia, in accordance with the latest views on the evolution of the vertebrates (Jarvik, 1960).

In the class of Crossopterygii, which is of special interest since it is regarded as the origin of land vertebrates, the two orders Coelacanthini and Dipnoi had otoliths of entirely different types. Thus, in the Coelacanthini, represented by *Latimeria chalumnae*, the static body examined was a polycrystalline statolith of the same structure and composition as found in teleosts. The lung-fish, *Protopterus*, on the other hand, had statoconia consisting of perfect single crystals of aragonite. This type of static bodies is otherwise found only in tetrapods. Although most modern taxonomists place the Coelacanthini and the Dipnoi in the same class, some scientists are not satisfied with this classification. Certain authorities regard the Dipnoi as an entirely separate class (Berg, 1958; Jarvik, 1960). This view has been adhered to in Figure 5. That many similarities exist between Dipnoi and Amphibia has been pointed out by Säve-Söderbergh (1934); there are, however, several dissimilarities which make a closer relationship between these groups improbable. Because of the restricted information available concerning the nature of the otoliths of crossopterygians we are unable here to draw any conclusions as to the significance of the present findings. Since coelacanths similar to *Latimeria* existed 400 million years ago, it seems probable that the Coelacanthini had developed at an early stage the highly specialized static bodies found in teleosts. Such otoliths are not likely to be the forerunners of the static bodies of land vertebrates. Therefore, paleozoological information on the type and structure of the otoliths of the primitive Rhipidistian fishes (Porolepiformes and Osteolepiformes), which are regarded as closest to the possible tetrapod ancestors (Jarvik, 1960), is badly wanted. It should not be too difficult to judge whether statoliths or statoconia were present in this group of fishes, as it can be anticipated that at least the size and shape of the otoliths should be very well preserved.

In the class of Amphibia, only statoconia in the form of single crystals of aragonite were found. From this point of view the amphibians form a completely homogeneous group. The curious composite "statoliths" of the axolotl, a larval form of a salamander, encourage further studies. It would be of special interest to know whether the larvae of lung-fishes possess similar otoliths.

The otoliths of Reptilia are interesting in that they seem to form an intermediate stage between amphibians on the one hand and birds and mammals on the other. In most species investigated, the statoconia consisted of a mixture of aragonite and calcite crystals. The highest percentage of calcite was found in the subclass Anapsida, which is supposed to be the living group closest related to mammals.

Finally, in the classes of birds and mammals, all species investigated possessed statoconia of the same nature, *viz.*, single crystals of calcite.

The correlation between the nature of the otoliths in different vertebrates and the commonly accepted classification is striking. Disagreements were found in a few instances only, and these were in fact restricted to groups where classification is still considered controversial. Even if the value of an investigation of this kind for the study of vertebrate phylogeny is limited, it is hoped that further studies, especially on paleozoological material, may aid in the classification of certain critical groups.

Apart from their possible phylogenetic significance, there are other interesting aspects of otoliths which certainly need further investigation. Why do vertebrates which have an abundance of calcium phosphate in their skeletons produce pure calcium carbonate in a single site in their body? From the functional point of view it would seem as if apatite, which has a higher density than any calcium carbonate, should be better suited for the function of the otolith organ. That apatite can be used instead of calcium carbonate is shown by the cyclostomes.

From studies on the domestic fowl it is known that the statoconia are deposited very early during development, even before the start of the calcification of the skeleton (Herzog, 1925). But it is not known if the statoconia, once formed, persist, or if there is a continuous production and perhaps also destruction of statoconia throughout the life-span of an animal.

Also, it is completely unknown why some vertebrates have more statoconia in their labyrinths than others. The total weight of the statoconia in a small ray or frog is thus many thousand times that of the statoconia of a man or a whale. In a frog, which has the endolymphatic sacs packed with statoconia, all these small bodies cannot possibly be needed for the function of the otolith organ.

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Summary

1. The crystallographic properties of otoliths from 58 vertebrate species were investigated by means of polarization microscopy and x-ray diffraction.

2. The otoliths occur as statoconia, microstatoliths, and statoliths. Usually only one kind is present in the labyrinth but in some vertebrates a combination of two or all three types may be found.

3. While statoliths and microstatoliths always are polycrystalline, statoconia may either be polycrystalline or single crystals.

4. Five different minerals, *viz.*, apatite, calcite, aragonite, vaterite and calcium carbonate monohydrate, compose the endogenous otoliths in the vertebrate labyrinth. Some elasmobranchs have in addition exogenous statoconia consisting of sea sand.

5. The distribution of statoliths and/or statoconia, their texture and their composition within the vertebrate series, show remarkable consistencies; within each class the same kind of static bodies is usually present.

6. Some crystallographic and biological aspects are discussed and it is suggested that the findings may be of some value for the study of the phylogeny of vertebrates and also may aid towards a better understanding of the function of the otolith organ.

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